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Sublethal effects of metaflumizone on abamectin-resistant and susceptible strains of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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Abstract: In order to provide the theoretical basis for the management of the diamondback moth, *Plutella xylostella* (L.) resistant to abamectin, the sublethal effects of metaflumizone on the experimental populations of both abamectin-resistant (AV-R) and susceptible (AV-S) strains of *P. xylostella* were evaluated by means of the life table technology. The results showed that the estimated LC_{50} and LC_{25} values of leaf dip bioassay of metaflumizone on the 3rd instar larvae were 0.24 and 0.09 mg/L for the AV-S strain, and 0.20 and 0.07 mg/L for the AV-R strain, respectively. Metaflumizone at the sublethal concentration (0.09 mg/L) tested decreased the pupation rate and pupal weight, prolonged the pupal duration and decreased the emergence rate, fecundity, oviposition duration and longevity of adults in the treated generation. Such treatment also decreased the hatchability and larval survival rate of the offsprings and their larval duration. Reproduction parameters such as the intrinsic rate of increase (r_m), finite rate of increase (λ), and the net reproductive rate (R_0) in populations treated with sublethal concentrations of metaflumizone were significantly lower than those of the control populations ($P < 0.0001$). The results also showed that the sublethal concentration of metaflumizone had a greater effect on the AV-R strain than on AV-S strain, and had a greater effect on the current generation than on the offsprings. We conclude that the sublethal concentration of metaflumizone might have significant effects on the population dynamics of *P. xylostella*, especially the AV-R strain, and metaflumizone might be extremely useful for managing pest resistance to insecticides.

Key words: *Plutella xylostella*; metaflumizone; abamectin; resistance strain; susceptible strain; sublethal effect; biological parameters

1 INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an important and worldwide pest of cruciferous vegetables (Talekar and Shelton, 1993). It has become a particularly notorious pest because of its resistance to almost every insecticide applied in the field (Sayyed *et al.*, 2004; Sarfraz and Keddie, 2005; Attique *et al.*, 2006; Zhao *et al.*, 2006). Up till now, *P. xylostella* has developed resistance to 81 insecticides including abamectin (Whalon *et al.*, 2012), which have been widely used to control *P. xylostella* in China. The resistance of *P. xylostella* to abamectin was about 5 000-fold higher in the field strain than in the susceptible laboratory strain (Roth) (Pu *et al.*, 2010). In order to control *P. xylostella* effectively, metaflumizone has been introduced.

Metaflumizone has been marketed globally for several years and registered for use in China against the larvae of *P. xylostella* on cabbage since 2010 (www.chinapesticide.gov.cn). Metaflumizone is a new semicarbazone insecticide which acts by blocking the voltage-dependant sodium channel of insect neurons (Klein and Oloumi, 2005). To our knowledge, metaflumizone does not require metabolic bio-activation by insects and has not been found to exhibit cross-resistance with existing insecticides such as organophosphates, carbamates, and benzoylphenylureas (Klein and Oloumi, 2005; Cutler *et al.*, 2006; Takagi *et al.*, 2007). Metaflumizone can control most lepidopterous pests and certain pests in such orders as Coleoptera,

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Hemiptera, Hymenoptera, Diptera, Isoptera and Siphonaptera effectively (Salgado and Hayashi, 2007). In addition, it has a low risk to non-target organisms including beneficial insects and pollinators, as well as humans and the environment (Hempel *et al.*, 2007; Gradish *et al.*, 2010).

Insecticides are usually applied to kill their target insects. However, target insects may experience sublethal doses because of poor spray coverage or the decrease of residuals after application (Wang *et al.*, 2008). Sublethal effects from exposure to insecticides on survivors have been shown to alter insect fecundity (Biddinger and Hull, 1999), developmental rate (Galvan *et al.*, 2005), behavior (El Hassani *et al.*, 2005) and appetite (Nauren *et al.*, 1998), and accelerate the development of insecticide resistance (Yin *et al.*, 2009). Additionally, hormoligosis may occur (Kerns and Stewart, 2000), which can result in stimulation of reproduction following contact of an insect with sublethal doses of insecticide (Kerns and Stewart, 2000). Cohen (2006) pointed out that the pest resurgence and further outbreak was mainly attributable to hormoligosis.

When making a pesticide selection, the sublethal effects must be considered together with mortality (Stark and Wennergren, 1995). Along with the large amount of metaflumizone being applied to control *P. xylostella*, the resistance to metaflumizone and the sublethal effects may occur in the future. Study of the sublethal effects of insecticides helps to correctly evaluate the efficiency in the field and coordinate chemical control and biological control. However, there are few reports on the lethal and sublethal effects of *P. xylostella* treated with metaflumizone, and little literature is available for comparison. In order to increase the use of metaflumizone in agriculture on the control of the resistant *P. xylostella*, it is necessary to study their sublethal effects. To explore the potential of metaflumizone for use in *P. xylostella* abamectin resistance management programs, we need to understand its sublethal effects on both suscepceptible and resistant population.

The total effect of pesticide at population levels can be evaluated by estimating the life table parameters such as the net reproductive rate (R_0), mean generation time (T) and intrinsic rate of increase (r_m) (Stark *et al.*, 1997; Maia *et al.*, 2000). The aim of this research is to provide the theoretical basis for management of *P. xylostella* resistance to abamectin through investigating the sublethal effects of metaflumizone on the biological characteristics of the abamectin-susceptible strain (AV-S) and the abamectin-resistant strain (AV-R) of *P. xylostella* under laboratory conditions.

2 MATERIALS AND METHODS

2.1 Test insects

The initial *P. xylostella* strain was collected from Xuanhua, Hebei province, China, in 1996, and reared continuously in the laboratory without exposure to insecticides for over 10 years as the abamectin-susceptible strain (AV-S). The abamectin-resistant strain (AV-R) was selected by abamectin from this susceptible strain. The larvae were reared routinely with vermiculite-cultured radish (*Raphanus sativus* L.) seedlings, and adults with 10% sugar solution as supplementary nutrition in the laboratory conditions of 27 ± 1℃, 70% - 80% relative humidity and a 16L∶8D photoperiod.

2.2 Insecticides

Abamectin (97.1% purity) was donated by North China Aino Co., Ltd., and metaflumizone (240 g/L SC) was supplied by BASF, Shanghai.

2.3 Bioassay and determination of sublethal concentration

The leaf dip bioassay method reported by Shelton *et al.* (1993) and Liang *et al.* (2003) was adopted for the toxicity bioassay of abamectin and metaflumizone. Cabbage leaves (6.5 cm in diameter) were cut and dipped in different concentrations of abamectin (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/L for the AV-S strain; 0, 20, 40, 80, 160 and 320 mg/L for the AV-R strain) and metaflumizone (0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L) solutions prepared with distilled water containing Triton X-100 (100 mg/L), respectively. Each disc was dipped for 10 s and allowed to air dry at room temperature. Then the discs were placed individually inside plastic Petri dishes (9.0 cm in diameter). Fifteen to twenty 3rd instar larvae were confined in each dish and three replications were prepared. Five or six concentrations and one control with distilled water containing 100 mg/L Triton X-100 were tested in each bioassay. Mortality was recorded at 48 h after treatment. The control mortality for all leaf dip bioassays was <5%.

2.4 Sublethal effects of metaflumizone on the treated generation of *P. xylostella*

The calculated LC_{25} values of the AV-S and AV-R strains of *P. xylostella* were very similar, and for this reason we applied LC_{25} (0.09 mg/L) of the AV-S strain to larvae of *P. xylostella* in this study. The test insects were divided into four populations:

AV-S (control), AV-S (LC_{25}) (the AV-S strain was treated with metaflumizone at LC_{25} concentration), and AV-R(control), AV-R(LC_{25}) (the AV-R strain was treated with metaflumizone at LC_{25} concentration).

Cabbage leaves treated with sublethal concentration (LC_{25}) of metaflumizone and the control were prepared with distilled water containing Triton X-100 (100 mg/L). The treated leaves were air-dried at room temperature. Thirty newly molted and physiological stage consistent 3rd instar larvae, which had been starved for 2 h, were placed on treated leaves in a plastic cup (7.0 cm at the opening and 5.0 cm at the bottom in diameter, and 8.5 cm in depth). For each treatment, seven replicates were set up. After 48 h, the surviving larvae were transferred to untreated cabbage leaves until their pupation. The leaves were changed when necessary and pupation was recorded daily. The 2-day-old pupae were removed and weighed individually using an analytical balance, and then placed into a small finger tube (1.2 cm in diameter, 5.5 cm in depth) until the adults emerged.

For the fecundity study, 30 newly emerged (0 – 12 h old) adult males and females were paired randomly in each treatment. Each pair was placed in a plastic cup (the same as above) and fed on cotton wool saturated with 10% honey solution. The open end of the cup was covered with cotton gauze. A piece of cabbage leaf (about 9.0 cm long × 6.0 cm wide) was inserted for oviposition. Leaves were replaced with fresh ones and numbers of eggs laid were recorded daily. This process continued until the death of the females.

2.5 Sublethal effects of metaflumizone on the offspring generation of *P. xylostella*

To study the growth and development of offsprings, 300 eggs (100 eggs per replicate) were randomly sampled from each treatment of the above experiment (section 2.4) and were confined in plastic cup (the same as above), and then incubated for hatching. The hatched larvae were placed individually in Petri dishes (9.0 cm in diameter) and supplied with fresh cabbage leaves. All insects were examined daily, and developmental duration and survival rate were recorded. The sexes of 4th instar larvae were determined using the method of Liu and Tabashnik (1997). Pupae were weighed individually as above. The method for counting the number of eggs was as explained above.

2.6 Data analysis

Bioassay data were analyzed by probit analysis using POLO software (LeOra Software, 1997). The data obtained from the experiments of sublethal effects were subjected to analysis of variance (ANOVA) and means were separated using Tukey's honest significant difference test at $P < 0.05$. Percentages were transformed to arcsine square root before analysis. The raw data of life history of all developmental stages of offsprings were used in the analysis of all life table parameters, which were then analyzed using a computer program, TWOSEX-MSChart (Chi, 2004). This program is available at http://nhsbig.inhs.uiuc.edu/wes/chi.html (the website of the Illinois Natural History Survey). Means and standard errors of biological parameters were estimated using the Jackknife method (Maia *et al.*, 2000). SPSS 11.0 software was used for all analysis.

3 RESULTS

3.1 Determination of sublethal concentration of metaflumizone to *P. xylostella*

The leaf dip bioassay showed that the LC_{50} value of abamectin to the AV-R strain was about 525.74 times higher than that to the AV-S strain (Table 1). However, the LC_{50} value of metaflumizone to the AV-S strain was 0.24 mg/L, and had no difference from the LC_{50} value (0.20 mg/L) to the AV-R strain, suggesting that there is no cross-resistance between abamectin and metaflumizone. The LC_{25} values of the AV-S strain and the AV-R strain were 0.09 mg/L and 0.07 mg/L, respectively. When the 3rd instar larvae were treated with this concentration (0.09 mg/L) for 48 h, the corrected mortality was 23.81% for the AV-S strain and 26.19% for the AV-R strain, respectively.

3.2 Sublethal effects of metaflumizone on pupae of the treated generation of *P. xylostella*

Sublethal concentration of metaflumizone had a considerable effect on pupae of the treated generation (Table 2). The pupal duration of treated populations were significantly prolonged than untreated populations when this pest was exposed to metaflumizone at the 3rd instar ($F = 95.079$, $df = 3,316$, $P < 0.0001$). The pupation rates and the adult emergence rates of the AV-S (LC_{25}) and AV-R (LC_{25}) populations were significantly lower than those of controls (pupation: $F = 10.896$, $df = 3, 24$, $P < 0.0001$; emergence: $F = 10.228$, $df = 3, 24$, $P < 0.0001$). There was no significant difference in the population rates and the adult emergence rates between the AV-S (control) and AV-R (control) populations, and the situation was the same between the AV-S (LC_{25}) and the AV-R

(LC_{25}) populations. Significant reduction in the mean pupal weight was also observed among the four treatments ($F = 294.760$, $df = 3, 604$, $P < 0.0001$).

Table 1 Toxicity of metaflumizone and abamectin to the 3rd instar larvae of *Plutella xylostella*

Strains	Insecticides	n[a]	Slope ± *SE*	LC_{50} (95% CI) (mg/L)	χ^2	RR	LC_{25} (95% CI) (mg/L)
AV-S	Abamectin	321	1.45 ± 0.18	0.31 (0.22 - 0.41)	9.29	1	-
AV-R	Abamectin	315	2.71 ± 0.40	162.98 (136.76 - 193.67)	12.36	525.74	-
AV-S	Metaflumizone	360	1.61 ± 0.17	0.24 (0.18 - 0.30)	8.28	1	0.09 (0.06 - 0.12)
AV-R	Metaflumizone	360	1.46 ± 0.16	0.20 (0.15 - 0.26)	21.88	0.83	0.07 (0.04 - 0.20)

[a] Number of larvae tested; CI: Confidence interval. RR: Resistance ratio.

Table 2 Effects of sublethal concentration of metaflumizone on pupae of the treated generation of *Plutella xylostella*

Treatments	Pupal duration (d)	Pupation rate (%)	Pupal weight (mg)	Emergence rate (%)
AV-S (control)	3.69 ± 0.07 c	82.38 ± 1.88 a	5.54 ± 0.04 a	82.70 ± 2.40 a
AV-R (control)	3.80 ± 0.08 c	76.19 ± 3.12 a	4.86 ± 0.04 b	80.26 ± 2.48 a
AV-S (LC_{25})	4.63 ± 0.07 b	67.62 ± 2.28 b	4.22 ± 0.06 c	68.25 ± 1.65 b
AV-R (LC_{25})	5.07 ± 0.06 a	63.81 ± 2.56 b	4.01 ± 0.09 c	62.84 ± 4.48 b
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	95.079	10.896	294.760	10.228
df	3,316	3,24	3,604	3,24

Values in the same column with different letters show significant difference (Tukey's HSD test) at the 0.05 level. The same for the following tables.

3.3 Sublethal effects of metaflumizone on adults of the treated generation of *P. xylostella*

The fecundity of the *P. xylostella* treated with metaflumizone were significantly lower than that of control populations ($F = 70.215$, $df = 3, 116$, $P < 0.0001$) (Table 3). It was worth noting that the number of eggs laid per female in the two controls was strikingly different, indicating that the biological performance of the two *P. xylostella* strains was different in the same environment. However, there was no significant difference between the AV-S (LC_{25}) and AV-R (LC_{25}) populations. In addition, metaflumizone reduced the longevity of adults and oviposition duration. The longevity and oviposition duration of females and males in the AV-R (LC_{25}) population were drastically shortened compared with the AV-S (control), AV-R (control) and AV-S (LC_{25}) populations (female: $F = 160.831$, $df = 3, 116$, $P < 0.0001$; male: $F = 77.287$, $df = 3, 116$, $P < 0.0001$; oviposition duration: $F = 54.369$, $df = 3, 116$, $P < 0.0001$).

Table 3 Effects of sublethal concentration of metaflumizone on adults of the treated generation of *Plutella xylostella*

Treatments	Number of eggs laid per female	Oviposition duration (d)	Mean longevity (d)	
			Female	Male
AV-S (control)	102.83 ± 5.57 a	5.77 ± 0.22 a	7.63 ± 0.12 a	9.63 ± 0.38 a
AV-R (control)	83.17 ± 3.01 b	4.90 ± 0.17 b	7.40 ± 0.13 a	9.17 ± 0.22 a
AV-S (LC_{25})	49.87 ± 2.89 c	5.10 ± 0.15 ab	6.77 ± 0.13 b	8.00 ± 0.19 b
AV-R (LC_{25})	37.03 ± 1.86 c	2.33 ± 0.26 c	3.23 ± 0.23 c	4.13 ± 0.31 c
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	70.215	54.369	160.831	77.287
df	3,116	3,116	3,116	3,116

The average number of eggs laid per female per day initially increased and then decreased (Fig. 1). The oviposition rate reached the peak at 1 d after the moths were paired and very few or no eggs were laid at

8 d. The same pattern was observed in each of the treatments. The number of eggs laid per female per day in the treated populations was much lower than that in the control populations.

3.4 Sublethal effects of metaflumizone on survival rate and development of the offsprings of *P. xylostella*

Survival rates of each life stage of *P. xylostella* exposed to LC_{25} dose of metaflumizone and controls are listed in Fig. 2. Survival rates in the two treatments were significantly lower than that in the untreated controls. The highest mortality was found at the 1st and 2nd larval instars. Survival rates in the AV-S and AV-R controls in each life stage were almost similar; however survival rates in the AV-S (LC_{25}) treatment at the 1st and 2nd instars were significantly higher than that in the AV-R (LC_{25}) treatment.

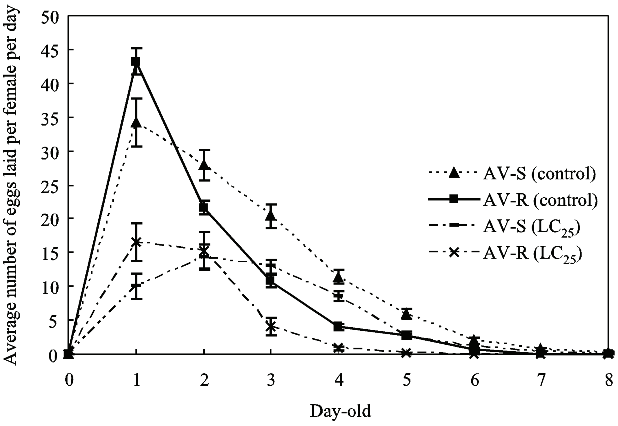

Fig. 1 Sublethal effects of metaflumizone on fecundity of *Plutella xylostella* female adults of different age after exposure of 3rd instar larvae to metaflumizone

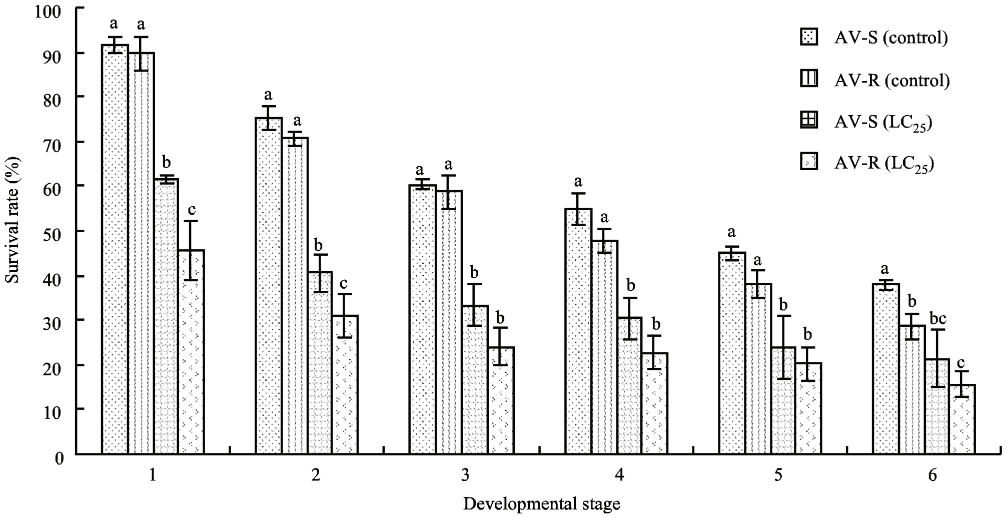

Fig. 2 Survival of *Plutella xylostella* offsprings in different treatments with metaflumizone

Life stage represents the stages from egg to adult emergence. Columns marked with different letters mean significant difference ($P \leqslant 0.05$) based on the least significant difference (Tukey's HSD) multiple comparison test. 1: 1st instar larva; 2: 2nd instar larva; 3: 3rd instar larva; 4: 4th instar larva; 5: Pupa; 6: Adult.

The effects of metaflumizone at sublethal concentration on developmental time of *P. xylostella* offsprings are presented in Table 4. There were significant differences in life span of the egg duration, larval duration, female preoviposition duration (APOD, the time between adult emergence and first oviposition) and total preoviposition duration (TPOD, the time from birth to first reproduction in the female) between the treatments and the controls (egg: $F = 16.20$, $df = 3, 1196$, $P < 0.0001$; larva: $F = 11.91$, $df = 3, 463$, $P < 0.0001$; APOD: $F = 28.97$, $df = 3, 154$, $P < 0.0001$; TPOD: $F = 39.76$, $df = 3, 154$, $P < 0.0001$). The total longevity of females in treatments was significantly different from that of the AV-S (control) population, but not significantly different from the AV-R (control) population. The egg duration, larval duration, pupal duration, total female longevity, APOD, TPOD of the AV-R (LC_{25}) population were significantly prolonged under the sublethal dose treatment. By contrast, adult longevity was shortened significantly. There were no significant differences in life span of the males between the treatments and the controls ($P > 0.05$).

3.5 Sublethal effects of metaflumizone on eggs, pupae and adults of the offsprings of *P. xylostella*

Metaflumizone had significant effects on egg hatching at sublethal dose (Table 5). The egg hatching rate in AV-S (LC_{25}) population decreased 1.49-fold compared with that in the AV-S (control) population, and that in the AV-R (LC_{25}) population decreased 1.96-fold compared with that in the AV-R (control) population. The pupation rate and pupal weight were similar between different treatments, except that the adult emergence rate of offsprings in treatments tended to be higher than that of the controls (Table 5). The average pupal weight of females in the AV-R (LC_{25}) population was 7.03 ± 1.29 mg, higher than that in other three populations, but there was no significant difference between them. The fecundity of the AV-R (LC_{25}) population was lower than that of other three populations, and had significant difference from that of the AV-S (control) population (Table 5).

Table 4 Effects of sublethal concentration of metaflumizone on developmental stage and longevity of the 3rd larvae of *Plutella xylostella*

Treatments	Developmental stage (d)			APOD (d)	TPOD (d)
	Egg	Larva	Pupa	Female	Female
AV-S(control)	3.24 ±0.05 b	7.86 ±0.08 c	3.48 ±0.05 c	0.52 ±0.02 b	14.25 ±0.13 d
AV-R(control)	3.27 ±0.04 b	8.13 ±0.11 bc	3.98 ±0.08 b	0.62 ±0.04 b	15.28 ±0.21 c
AV-S(LC_{25})	3.52 ±0.04 a	8.53 ±0.12 ab	4.04 ±0.08 b	0.88 ±0.05 a	16.06 ±0.22 b
AV-R(LC_{25})	3.70 ±0.07 a	8.72 ±0.14 a	4.52 ±0.10 a	0.93 ±0.06 a	17.30 ±0.28 a
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F	16.20	11.91	31.96	28.97	39.76
df	3,1 196	3,463	3,378	3,154	3,154

Treatments	Adult longevity (d)		Total longevity (d)	
	Female	Male	Female	Male
AV-S(control)	7.61 ±0.19 a	10.52 ±0.34 a	21.34 ±0.24 b	25.05 ±0.37 a
AV-R(control)	7.56 ±0.21 a	10.15 ±0.23 a	22.22 ±0.28 ab	25.17 ±0.35 a
AV-S(LC_{25})	7.88 ±0.20 a	9.52 ±0.30 a	23.06 ±0.30 a	25.54 ±0.38 a
AV-R(LC_{25})	5.95 ±0.40 b	9.30 ±0.53 a	23.27 ±0.49 a	26.26 ±0.59 a
P	<0.0001	0.056	<0.0001	0.228
F	9.16	2.57	8.79	1.46
df	3,154	3,158	3,154	3,158

Means within the same column followed by different letters are significantly different ($P < 0.05$; Scheffe mean separation). APOD: Adult preoviposition duration, time between adult emergence and first oviposition; TPOD: Total preoviposition duration, time from birth to first reproduction in female.

Table 5 Effects of sublethal concentration of metaflumizone on eggs, pupae and adults of the offsprings of *Plutella xylostella*

Treatments	Egg hatch rate (%)	Pupation rate (%)	Pupal weight (mg)		Emergence rate (%)	Number of eggs laid per female
			Female(± *SE*)	Male(± *SE*)		
AV-S (control)	91.67 ±1.76 a	82.05 ±4.14 a	6.28 ±0.09 a	5.17 ±0.05 a	88.30 ±2.84 a	104.49 ±6.15 a
AV-R (control)	89.67 ±3.76 a	79.51 ±2.65 a	5.52 ±0.14 a	4.91 ±0.10 a	76.98 ±1.98 a	92.13 ±2.71 ab
AV-S (LC_{25})	61.67 ±0.88 b	79.47 ±2.57 a	6.16 ±0.15 a	4.84 ±0.19 a	88.64 ±1.98 a	76.12 ±9.88 ab
AV-R (LC_{25})	45.67 ±6.69 b	89.69 ±4.04 a	7.03 ±1.29 a	4.93 ±0.11 a	79.91 ±3.61 a	69.36 ±8.91 b
P	<0.0001	0.161	0.137	0.129	0.706	0.039
F	32.897	2.237	1.866	1.913	0.479	4.524
df	3,8	3,8	3,165	3,177	3,8	3,8

3.6 Sublethal effects of metaflumizone on population growth parameters of *P. xylostella*

Effects of sublethal concentration of metaflumizone on life table parameters of *P. xylostella* are listed in Table 6. The mean values of the intrinsic rate of increase (r_m), finite rate of increase (λ), the net reproductive rate (R_0) of the AV-S (LC_{25}) and AV-R (LC_{25}) populations tended to be lower than those of the controls and the differences were significant ($P < 0.0001$). The R_0 of the AV-R (LC_{25}) population was reduced about 78.93% and 60.79% compared with the AV-S (control) and AV-R (control) populations, respectively. The generation time (T) and the doubling time (D_t) of the AV-S (LC_{25}) and AV-R (LC_{25}) populations were significantly longer than that of the controls. The values of r_m, λ, T and the gross reproductive rate (*GRR*) in the AV-S (LC_{25}) and AV-R (LC_{25}) populations had no significant difference, but *GRR* in the AV-S (LC_{25}) and AV-R (LC_{25}) populations was significantly lower than that of the AV-S (control) population.

Table 6 Effects of sublethal concentration of metaflumizone on life table parameters of the offsprings after treatment in the 3rd instar larvae of *Plutella xylostella*

Treatments	r_m	λ	*GRR*	R_0	T (d)	t (d)
AV-S (control)	0.19 ± 0.01 a	1.21 ± 0.01 a	55.90 ± 0.60 a	23.54 ± 0.26 a	16.88 ± 0.07 b	3.68 ± 0.02 c
AV-R (control)	0.15 ± 0.01 b	1.15 ± 0.01 b	42.59 ± 3.23 ab	12.65 ± 1.48 b	17.66 ± 0.17 ab	4.80 ± 0.24 bc
AV-S (LC_{25})	0.12 ± 0.02 bc	1.13 ± 0.02 bc	39.57 ± 4.91 b	8.57 ± 1.95 bc	18.67 ± 0.61 ab	6.09 ± 0.69 b
AV-R (LC_{25})	0.09 ± 0.02 c	1.08 ± 0.02 c	31.42 ± 3.97 b	4.96 ± 0.41 c	19.85 ± 0.78 a	8.10 ± 0.13 a
P	< 0.0001	< 0.0001	0.008	< 0.0001	0.015	< 0.0001
F	26.96	31.08	8.20	41.59	6.53	28.07
df	3,1 196	3,1 196	3,1 196	3,1 196	3,1 196	3,1 196

4 DISCUSSION

Results of the present study show that metaflumizone is a highly active insecticide against *P. xylostella*. The LC_{50} values of metaflumizone to 3rd instar larvae of the AV-S and AV-R strains (525.74-fold) at 48 h after treatment were as low as 0.24 mg/L and 0.20 mg/L, respectively. We confirmed that metaflumizone not only has no known cross-resistance to abamectin, but also proves highly effective in control of abamection-resistant populations. Abamectin was introduced as a new insecticide for *P. xylostella* control in 1995, and it was widely and intensively used because of its high potency (Zhang *et al.*, 2001). Now, it has become ineffective in controlling *P. xylostella* in the field in recent years (Pu *et al.*, 2010).

In order to make good use of metaflumizone to control *P. xylostella* with resistance to abamectin, the present study revealed numerous sublethal effects of the insecticide on *P. xylostella* at the population level, except for the toxic effect. Metaflumizone at sublethal concentration had a considerable impact on biological characteristics of *P. xylostella*. The results presented here showed that the sublethal effects of metaflumizone on biological parameters were greater in the AV-R (LC_{25}) population than in the AV-S (LC_{25}) population.

The results in this experiment demonstrated that sublethal concentration of metaflumizone in the treated current generation reduced pupal weight of *P. xylostella*, but the offsprings were not affected by metaflumizone, indicating that the direct effect of metaflumizone was greater than the indirect effect. This is because metaflumizone is gradually degraded with time after being applied. However, the pupal weight tends to increase in offsprings of the AV-R (LC_{25}) population. There is no sufficient evidence to support this phenomenon, so more investigations are needed.

In this study, it was found that treatment of sublethal concentration of metaflumizone at the larval stage shortened adult longevity and oviposition duration, and reduced fecundity and egg hatchability. In the offsprings, the fecundity also diminished. Decreasing fecundity and hatchability are very beneficial factors to successful pest management. It was also found that metaflumizone at sublethal doses could cause a decrease in survival and delay larval development of the offsprings.

Demographic toxicology has become quite an accepted approach, whereby the life table parameters can elucidate the total effects of toxins at the population level (Stark and Wennergren, 1995; Walthall and stark, 1997). In this study, the larval duration, pupal duration, total adult longevity of females, adult preoviposition duration and total preoviposition duration tended to be longer in the treatments than those in the controls. Therefore, the mean generation time (T) of *P. xylostella* was prolonged. The mean values of intrinsic rate of increase (r_m), finite rate of increase (λ), gross reproduction rate (*GRR*) and net reproductive rate (R_0) tended to be lower in treatments than those in the controls. Similar results were obtained by Yin *et al.* (2008), who reported that sublethal doses of spinosad (LC_{25} and LC_{50}) decreased r_m, R_0 and *GRR*, and increased *Dt* in *P. xylostella*. Mahmoudvand *et al.* (2011) indicated that LC_{10} and LC_{25} concentrations of hexaflumuron significantly increased *Dt*, decreased R_0 and r_m, and LC_{10}

concentration increased *GRR* in *P. xylostella*.

In the case of *P. xylostella*, insecticide hormoligosis has been previously reported in insects treated with fenvalerate (Fujiwara *et al.*, 2002), hexaflumuron (Mahmoudvand *et al.*, 2011) and beta-cypermethrin (Han *et al.*, 2011). Yin *et al.* (2009) stated that the phenomenon of reproduction of *P. xylostella* by exposure of successive generations to the sublethal dose (LC_{25}) of spinosad might result in the resurgence of *P. xylostella* and further outbreaks of the pest population. But in this study, insecticide hormoligosis was not observed when *P. xylostella* was treated with metaflumizone at LC_{25}, in both AV-S and AV-R strains. Additional studies need to focus on the efficacy of sublethal concentration of metaflumizone on successive generations of *P. xylostella*.

The present study has also demonstrated that AV-R strain had a relatively lower fitness as compared with the AV-S strain. Similar result was found by Li *et al.* (1999). This means that resistance would be effectively decreased when insects are bred free from this insecticide. Pu *et al.* (2010) also reported that abamectin could be reused after several generations of suspension because a field-evolved high-level resistance to abamectin is unstable in the TH population of *P. xylostella* (the population was collected from Tonghai County, Yunnan Provine, China). Thus we not only can use metaflumizone to control the AV-R starin of *P. xylostella*, but also can prolong service life of abamectin by the rotation with metaflumizone.

In summary, metaflumizone has lethal and sublethal effects on *P. xylostella*, which may affect population dynamics in the field via reduction in survival and reproduction. Metaflumizone will be extremely useful for managing resistance to insecticides. The novel risk-reduced insecticide metaflumizone was harmless to *Orius insidiosus* (Say), *Amblyseius swirskii* (Athias-Henriot) and *Eretmocerus eremicus* (Rose & Zolnerovich) (Gradish *et al.* 2011). Gradish *et al.* (2010) found that metaflumizone had no sublethal effects on bumble bees. Therefore, future study is required to determine if metaflumizone has sublethal effects on natural enemies of *P. xylostella*.

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氰氟虫腙对小菜蛾阿维菌素抗性和敏感种群的亚致死效应

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摘要: 在实验室条件下采用生命表技术研究了氰氟虫腙亚致死剂量(LC_{25})对小菜蛾 *Plutella xylostella* 阿维菌素抗性(AV-R)和敏感(AV-S)种群的亚致死效应, 旨在为小菜蛾对阿维菌素的抗性治理提供理论基础。结果表明: 氰氟虫腙对小菜蛾3龄幼虫抗性种群的 LC_{50} 和 LC_{25} 分别为 0.24 mg/L 和 0.09 mg/L; 对敏感种群的 LC_{50} 和 LC_{25} 分别为 0.20 mg/L 和 0.07 mg/L。氰氟虫腙亚致死剂量 0.09 mg/L 处理小菜蛾后, 对处理代的影响表现为显著降低处理种群的化蛹率、蛹重、羽化率、繁殖力; 明显延长蛹期, 缩短成虫产卵期和寿命; 对子代种群的影响表现为显著降低卵的孵化率、幼虫各龄期的存活率, 延长发育历期。处理种群的内禀增长率(r_m)、周限增长率(λ)和净增值率(R_0)显著低于对照种群($P < 0.0001$)。亚致死剂量的氰氟虫腙对小菜蛾抗性种群的影响大于敏感种群, 对处理代种群的影响大于子代种群。氰氟虫腙亚致死剂量可以极大地影响小菜蛾尤其是阿维菌素抗性种群的种群动态, 因此氰氟虫腙对于小菜蛾的抗性治理具有积极的作用。

关键词: 小菜蛾; 氰氟虫腙; 阿维菌素; 抗性种群; 敏感种群; 亚致死效应; 生物学参数

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